

IN THE SPECIFICATION

Please amend the specification as shown:

Please delete the paragraph on page 26, lines 31-32, and replace it with the following paragraph:

[0001] **Figure 23** shows a BLAST Sequence Comparision of known and hypothetical mannosidase II, mannosidase IIx and Class III mannosidases **(SEQ ID NOS 96, 92, 93, 99, 98, 97, 94, 95, and 100-102, respectively in order of appearance).**

Please delete the paragraph on page 27, lines 2-3, and replace it with the following paragraph:

[0002] **Figure 25** shows an *Arabidopsis thaliana* Mannosidase II (NM_121499) **nucleotide** Sequence **(SEQ ID NO: 49) and encoded protein (SEQ ID NO: 95).**

Please delete the paragraph on page 27, line 4, and replace it with the following paragraph:

[0003] **Figure 26** shows a *C. elegans* Mannosidase II (NM_073594) **nucleotide** Sequence **(SEQ ID NO: 50) and encoded protein (SEQ ID NO: 92).**

Please delete the paragraph on page 27, lines 5-6, and replace it with the following paragraph:

[0004] **Figure 27** shows a *Ciona intestinalis* mannosidase II (AK116684) **nucleotide** Sequence **(SEQ ID NO: 51) and encoded protein (SEQ ID NO: 94).**

Please delete the paragraph on page 27, line 7, and replace it with the following paragraph:

[0005] Figure 28 shows a *D. melanogaster* mannosidase II (X77652) nucleotide Sequence (SEQ ID NO: 52) and encoded protein (SEQ ID NO: 96).

Please delete the paragraph on page 27, line 8, and replace it with the following paragraph:

[0006] Figure 29 shows a human mannosidase II (U31520) nucleotide Sequence (SEQ ID NO: 53) and encoded protein (SEQ ID NO: 97).

Please delete the paragraph on page 27, line 9, and replace it with the following paragraph:

[0007] Figure 30 shows a mouse mannosidase II (X61172) nucleotide Sequence (SEQ ID NO: 54) and encoded protein (SEQ ID NO: 98).

Please delete the paragraph on page 27, line 10, and replace it with the following paragraph:

[0008] Figure 31 shows a rat mannosidase II (XM_218816) nucleotide Sequence (SEQ ID NO: 55) and encoded protein (SEQ ID NO: 93).

Please delete the paragraph on page 27, line 11, and replace it with the following paragraph:

[0009] Figure 32 shows a human mannosidase IIx (D55649) nucleotide Sequence (SEQ ID NO: 56) and encoded protein (SEQ ID NO: 99).

Please delete the paragraph on page 27, line 12, and replace it with the following paragraph:

[0010] Figure 33 shows an insect cell mannosidase III (AF005034) nucleotide Sequence (SEQ ID NO: 57) and encoded protein (SEQ ID NO: 100).

Please delete the paragraph on page 27, lines 13-14, and replace it with the following paragraph:

[0011] Figure 34 shows a human lysosomal mannosidase II (NM_000528) nucleotide Sequence (SEQ ID NO: 58) and encoded protein (SEQ ID NO: 101).

Please delete the paragraph on page 27, lines 15-16, and replace it with the following paragraph:

[0012] Figure 35 shows a human cytoplasmic mannosidase II (NM_006715) nucleotide Sequence (SEQ ID NO: 59) and encoded protein (SEQ ID NO: 102).

Please delete Table 2 on page 46, and replace it with the following Table:

Table 2. PCR Primers

<u>PCR primer A</u>	<u>PCR primer B</u>	<u>Target Gene(s) in</u> <i>P.pastoris</i>	<u>Homologs</u>
ATGGCGAAGGC AGATGGCAGT (SEQ ID NO: 18)	TTAGTCCTTC CAACTTCCTT C (SEQ ID NO: 19)	1,6-mannosyltransferase	OCH1 <i>S.cerevisiae</i> , <i>Pichia albicans</i>
TAYTGGMGNGT NGARCYNGAY ATHAA (SEQ ID NO: 103)	GCRTCNCCCC ANCKYTCRTA (SEQ ID NO: 104)	1,2-mannosyltransferases	KTR/KRE family, <i>S.cerevisiae</i>

Legend: M = A or C, R = A or G, W = A or T, S = C or G,
Y = C or T, K = G or T, V = A or C or G, H = A or C or T, D = A or G or T, B = C or G or T,
N = G or A or T or C.

Please delete the paragraph on page 98, lines 1-11, and replace it with the following paragraph:

[0013] Another useful sub-library includes nucleic acid sequences encoding targeting signal peptides that result in localization of a protein to a particular location within the ER, Golgi, or trans Golgi network. These targeting peptides may be selected from the host organism to be engineered as well as from other related or unrelated organisms. Generally such sequences fall into three categories: (1) N-terminal sequences encoding a cytosolic tail (ct), a transmembrane domain (tmd) and part or all of a stem region (sr), which together or individually anchor proteins to the inner (luminal) membrane of the Golgi; (2) retrieval signals which are generally found at the C-terminus such as the HDEL **(SEQ ID NO: 105)** or KDEL tetrapeptide **(SEQ ID NO: 106)**; and (3) membrane spanning regions from various proteins, e.g., nucleotide sugar transporters, which are known to localize in the Golgi.

Please delete the paragraph on page 98, lines 21-27, and replace it with the following paragraph:

[0014] Still other useful sources of targeting peptide sequences include retrieval signal peptides, e.g. the tetrapeptides HDEL (SEQ ID NO: 105) or KDEL (SEQ ID NO: 106), which are typically found at the C-terminus of proteins that are transported retrograde into the ER or Golgi. Still other sources of targeting peptide sequences include (a) type II membrane proteins, (b) the enzymes listed in **Table 3**, (c) membrane spanning nucleotide sugar transporters that are localized in the Golgi, and (d) sequences referenced in **Table 5**.

Please delete Table 5 on pages 98-100, and replace it with the following Table:

Table 5. Sources of useful compartmental targeting sequences

<i>Gene or Sequence</i>	<i>Organism</i>	<i>Function</i>	<i>Location of Gene Product</i>
<i>MNSI</i>	<i>A.nidulans</i>	α -1,2-mannosidase	ER
<i>MNSI</i>	<i>A.niger</i>	α -1,2-mannosidase	ER
<i>MNSI</i>	<i>S.cerevisiae</i>	α -1,2-mannosidase	ER
<i>GLSI</i>	<i>S.cerevisiae</i>	glucosidase	ER
<i>GLSI</i>	<i>A.niger</i>	glucosidase	ER
<i>GLSI</i>	<i>A.nidulans</i>	glucosidase	ER
HDEL (<u>SEQ ID NO: 105</u>) at C-terminus	<i>Universal in fungi</i>	retrieval signal	ER
<i>SEC12</i>	<i>S.cerevisiae</i>	COPII vesicle protein	ER/Golgi
<i>SEC12</i>	<i>A.niger</i>	COPII vesicle protein	ER/Golgi
<i>OCH1</i>	<i>S.cerevisiae</i>	1,6-mannosyltransferase	Golgi (cis)
<i>OCH1</i>	<i>P.pastoris</i>	1,6-mannosyltransferase	Golgi (cis)
<i>MNN9</i>	<i>S.cerevisiae</i>	1,6-mannosyltransferase complex	Golgi
<i>MNN9</i>	<i>A.niger</i>	undetermined	Golgi
<i>VAN1</i>	<i>S.cerevisiae</i>	undetermined	Golgi

<i>Gene or Sequence</i>	<i>Organism</i>	<i>Function</i>	<i>Location of Gene Product</i>
<i>VAN1</i>	<i>A.niger</i>	undetermined	Golgi
<i>ANP1</i>	<i>S.cerevisiae</i>	undetermined	Golgi
<i>HOCl</i>	<i>S.cerevisiae</i>	undetermined	Golgi
<i>MNN10</i>	<i>S.cerevisiae</i>	undetermined	Golgi
<i>MNN10</i>	<i>A.niger</i>	undetermined	Golgi
<i>MNN11</i>	<i>S.cerevisiae</i>	undetermined	Golgi (cis)
<i>MNN11</i>	<i>A.niger</i>	undetermined	Golgi (cis)
<i>MNT1</i>	<i>S.cerevisiae</i>	1,2-mannosyltransferase	Golgi (cis, medial
<i>KTR1</i>	<i>P.pastoris</i>	undetermined	Golgi (medial)
<i>KRE2</i>	<i>P.pastoris</i>	undetermined	Golgi (medial)
<i>KTR3</i>	<i>P.pastoris</i>	undetermined	Golgi (medial)
<i>MNN2</i>	<i>S.cerevisiae</i>	1,2-mannosyltransferase	Golgi (medial)
<i>KTR1</i>	<i>S.cerevisiae</i>	undetermined	Golgi (medial)
<i>KTR2</i>	<i>S.cerevisiae</i>	undetermined	Golgi (medial)
<i>MNN1</i>	<i>S.cerevisiae</i>	1,3-mannosyltransferase	Golgi (trans)
MNN6	<i>S.cerevisiae</i>	Phosphomannosyltransferase	Golgi (trans)
2,6 ST	<i>H. sapiens</i>	2,6-sialyltransferase	trans Golgi network
UDP-Gal T	<i>S. pombe</i>	UDP-Gal transporter	Golgi

Please delete the paragraph on page 142, lines 14-22, and replace it with the following paragraph:

[0015] Restriction and modification enzymes were from New England BioLabs (Beverly, MA). The shuttle vector pVM2 was generated from pUC19 by inverse PCR (Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) In: Molecular Cloning, a Laboratory Manual 2nd Edition, Cold Spring Harbor N.Y.: Cold Spring Harbor Laboratory Press.) using the primers

VJM104 and VJM106 (5'- GCGGCCGCGGATCCCCGGGTACCGAGCTCGAATTCAC-
3' **(SEQ ID NO: 107)** and 5'- GGGGCGCGCC
TTAATTAACGACCTGCAGGCATGCAAGCTTGGCGTAATCATGGTCAT-3' **(SEQ ID
NO: 108)** respectively, introduced restriction sites NotI, AscI and PacI are underlined).

Please delete the paragraph on page 142, line 23, to page 143, line 30, and replace it with the following paragraph:

[0016] The roll-in plasmid pJN285 is a derivative of the knock-in plasmid pJN266 that was constructed in the following way. A 0.9-kb fragment of the *PpKEX1*-5' region was amplified by PCR using primers Kex55 (5'-

GGCGAGCTCGGCCTACCCGGCCAAGGCTGAGATCATTGTCCAG

CTTCAGA -3', **SEQ ID NO: 27)** and Kex53 (5'-

GCCCACGTCGACGGATCCGTTTAAACATCGATTGGAG

AGGCTGACACCGCTACTA-3', **SEQ ID NO: 28)** from *Pichia pastoris* genomic DNA and cloned into pUC19 digested with *SacI* and *SalI*. The resulting plasmid was cut with *BamHI* and *SalI*, and a 0.8-kb fragment of the *KEX1*-3' region that had been amplified using primers Kex35 (5'-

CGGGATCCACTAGTATTTAAATCATATGTGCGAGTGTAACAACCTTCCCACATGG-
3', **SEQ ID NO: 29)** and Kex33 (5'-

GGACGCGTCGACGGCCTACCCGGCCGTACGAGGAATTTCTCGGATGA

CTCTTTTC -3', **SEQ ID NO: 30)** was cloned into pJN262 digested with the same enzymes.

This plasmid was cut with *BamHI* and the 3.8-kb *BamHI*-*BglII* fragment of pNKY51 (1) was inserted in each of the two possible orientations resulting in plasmids pJN263 and pJN264.

To create an expression cassette with *NotI* and *PacI* cloning sites, the *GAPDH* promoter of *P. pastoris* was amplified using primers Gap5 (5'-

CGGGATCCCTCGAGAGATCTTTTTGTAGAAATGTCTTGGTGCCT -3', **SEQ ID
NO: 31)** and Gap3 (5'-

GGACATGCATGCACTAGTGCGGCCGCCACGTGATAGTTGTTCA

ATTGATTGAAATAGGGACAA -3', SEQ ID NO: 32) and plasmid pGAPZ-A (Invitrogen) as template and cloned into the *Bam*HI-*Sph*I sites of pUC19. The resulting plasmid was cut with *Spe*I and *Sph*I and the *S. cerevisiae* *CYC1* transcriptional terminator region, that had been amplified from pPICZ-A (Invitrogen) using primers Cyc5 (5'-

CCTTGCTAGCTTAATTAACC

GCGGCACGTCCGACGGCGGCCCCACGGGTCCCA -3', SEQ ID NO: 33) and Cyc3 (5'-GGACATGCATG

CGGATCCCTTAAGAGCCGGCAGCTTGCAAATTAAAGCCTTCGAGCGTCCC -3',

SEQ ID NO: 34), was cloned into the open sites creating pJN261. The *GAPDH/CYC1* expression cassette was released by *Bam*HI digestion and cloned either into pJN263 resulting in plasmid pJN265, or into pJN264 resulting in plasmids pJN266 and pJN267 (depending on orientation of the insert). Subsequently the plasmid pJN266 was cut with *Ngo*MIV and *Swa*I to release the URA-blaster cassette, and a *Ngo*MIV-*Swa*I fragment containing the *PpHIS4* gene, that had been amplified from pPIC3.5 (Invitrogen) using primers JNHIS1 (5'-

GCCCAAGCCGGCCTTAAGGGATCTCCTGAT

GACTGACTCACTGATAATAAAAATACGG-3', SEQ ID NO: 39) and JNHIS2 (5'-

GGGCGCGTATTTAAA

TACTAGTGGATCTATCGAATCTAAATGTAAGTTAAAATCTCTAA-3', SEQ ID NO: 40), was cloned into the open sites to create pJN285.

Please delete the paragraph on page 143, line 31, to page 144, line 7, and replace it with the following paragraph:

[0017] The pJN348 expression vector is based on plasmid pBLURA-SX (2). First a *Bam*HI fragment containing the *GAPDH/CYC1* expression cassette from vector pJN261 was cloned into pBLURA-SX that had been cut with *Bam*HI and *Bgl*II to create plasmid pJN338. Subsequently the latter plasmid was cut with *Not*I and *Pac*I and the two oligonucleotides Expr1 (5'-GGCCGCCTGCAGATTTAAATGAATTCGGCGCGCCTTAAT-3', SEQ ID NO: 41) and Expr2 (5'-TAAGGCGCGCCGAATTCATTAAATCTGCAGGGC-3' (SEQ ID

NO: 42), the restriction site *AscI* is underlined) that had been annealed *in vitro*, were ligated into the open sites, to create pJN348.

Please delete the paragraph on page 144, lines 8-20, and replace it with the following paragraph:

[0018] The pPB124 expression vector was constructed in several steps based on pBLADE-SX vector described by Cereghino et al. *Gene* 263 (2001) 159-169. First, *BamHI* fragment containing GAPDH/CYC1 expression cassette from vector pJN261 (described in Choi et al. *Proc Natl Acad Sci U S A.* 2003 Apr 29;100(9):5022-7) was cloned into pBLADE-SX vector after *BamHI*-*BglII* digest. Next, the *XhoI*-*NotI* fragment containing *P. pastoris* GAPDH promoter was replaced with the promoter of *P. pastoris PMA1* gene that was amplified with PMA1 (5'-TTCCTCGAGATTCAAGCGAATGAGAATAATG-3', **SEQ ID NO: 109**) and PMA2 (5'-TTGCGGCCGCGAAG TTTTAAAGGAAAGAGATA-3', **SEQ ID NO: 110**) primers. The resulting vector was then digested with *XbaI*-*BamHI* enzymes to remove *ADE1* marker, and after fill-in reaction ligated with blunt-ended *BglII*-*SacI* fragment containing nourseothricin resistance marker from vector pAG25 (Goldstein and McCusker, *Yeast.* 1999 Oct;15(14):1541-53).

Please delete the paragraph on page 144, line 24, to page 145, line 9, and replace it with the following paragraph:

[0019] *Amplification of mouse mannosidase IA.* The gene sequence encoding the catalytic domain of mouse mannosidase IA (Genbank: NM_008548, Lal & Moremen 1994) was amplified from mouse liver cDNA (Clontech). Briefly, the forward primer mMIAΔ187-*AscI* and reverse primer mMIA-*PacI* (5'-
GGCGCGCCGAGCCCGCTGACGCCACCATCCGTGAGAAGAGG GC-3' (**SEQ ID NO: 111**) and 5'-
CCTTAATTAATCATTTCTCTTTGCCATCAATTCCTTCTTCTGTTCACGG-3' (**SEQ ID NO: 26**), respectively, introduced *AscI* and *PacI* restriction sites are underlined) where

used to amplify amino acids 188-655 of the mouse mannosidase IA ORF from mouse liver cDNA (Clontech) with Pfu DNA polymerase (Stratagene). The conditions used for thermo cycling were: 94°C for 1min, 1 cycle; 94°C for 30 sec, 68°C for 30sec, 72°C for 3min, 30 cycles. Subsequently, 1µl *Taq* DNA polymerase (Promega) was added and the reaction further incubated at 72°C for 10min with the 1.4Kb product being ligated into pCR2.1, giving the plasmid pSH9. Following confirmation of the PCR product by *Taq* DyeDeoxy terminal sequencing the mouse mannosidase IA was digested with the restriction enzymes *AscI* and *PacI* prior to subcloning into the vector pVM2, digested with the same restriction enzymes, generating the plasmid pSH21.

Please delete the paragraph on page 145, lines 10-25, and replace it with the following paragraph:

[0020] To facilitate the subsequent localization of the truncated mouse mannosidase IA to the yeast Golgi a region of the *S.cerevisiae* Sec12 protein (amino acids 331-432, encoding the transmembrane domain) was amplified with the primers SC125 and SC122 (5'-ATGTGGCGGCGGCCCGCCACCATGAACACTATCCACATAATAAAATTACCGCTTAACTACGCC-3' (**SEQ ID NO: 112**) and 5'-GGCGCGCCCCACGCCTAGCACTTTTATGGAATCTACGCTAGGTAC-3' (**SEQ ID NO: 113**), respectively, introduced *NotI* and *AscI* restriction sites are underlined) in the presence of *Taq* DNA polymerase and *S.cerevisiae* genomic DNA, producing the plasmid pJN305. Following confirmation of the PCR product by *Taq* DyeDeoxy terminal sequencing the *Sec12* fragment, digested with the restriction enzymes *NotI* and *AscI*, was subcloned into pSH21 digested with the same enzymes, generating the plasmid pSH29. Subsequently the *NotI/PacI* fragment of pSH29, encoding the *Sec12* fragment in-frame with the truncated mouse mannosidase IA, was subcloned into pJN285 digested with the same enzymes, generating the plasmid pFB8.

Please delete the paragraph on page 145, line 28, to page 146, line 8, and replace it with the following paragraph:

[0021] The catalytic domain of a *Drosophila* mannosidase II (GenBank: X77652, Foster and Roberts 1995), encoding amino acids 75-1108, was amplified from *Drosophila* ovary cDNA using *ExTaq* DNA polymerase under the thermocycling conditions outlined above, by annealing at 55°C and extending for 5 minutes. The forward primer dMannIIΔ74_*Asc*I and the reverse primer dMannII_*Pac*I (5'-

GGCGCGCCCGCGACGATCCAATAAGACCTCCAC-3' (SEQ ID NO: 69) and 5'-
CCTTAATTAATCAGCTTG AGTGACTGCTCACATAAGCGGCGG-3' (SEQ ID NO: 71), respectively, introduced *Asc*I and *Pac*I restriction sites are underlined) were used.

Following confirmation of the PCR product by *Taq* DyeDeoxy terminal sequencing, the plasmid was named pSH214. Subsequently, the *Drosophila* mannosidase II fragment was removed from this plasmid by digestion with the restriction enzymes *Asc*I and *Pac*I, and subcloned into pJN348 digested with the same enzymes, generating the plasmid pSH220.

Please delete the paragraph on page 146, lines 9-24, and replace it with the following paragraph:

[0022] To facilitate the subsequent localization of the truncated *Drosophila* mannosidase II domain to the Golgi, a region of the *S.cerevisiae* Mnn2 protein (amino acids 1-36, encoding the transmembrane domain) was amplified with the primers Mnn25 and Mnn21 (5'-
AGTAAAATGCGGCCCGCCACCATGCTGCTTACCAAAGGTTTTCAAAGCTGTTC-3'
(SEQ ID NO: 114) and 5'-

GGCGCGCCCCGACGTGTTCTCATCCATGTATTTGTTTGTAATGAC-3' (SEQ ID NO: 115), respectively, introduced NotI and *Asc*I restriction sites are underlined) in the presence of *Taq* DNA polymerase and *S.cerevisiae* genomic DNA, producing the plasmid pJN281. Following confirmation of the PCR product by *Taq* DyeDeoxy terminal sequencing, the *Mnn2* fragment was digested with the restriction enzymes NotI and *Asc*I and subcloned into pSH220 digested with the same enzymes, producing an in-frame fusion of the *Mnn2* localization signal with the *Drosophila* mannosidase II catalytic domain, generating the

plasmid pKD53. The pH optimum of this engineered *Drosophila* mannosidase II catalytic domain was determined to be pH 6.2 using a pH assay essentially as described in **Example 7**.

Please delete the paragraph on page 150, lines 1-19, and replace it with the following paragraph:

[0023] The construction of a GnTI expression vector (pNA15) containing a human GnTI gene fused with the N-terminal part of *S. cerevisiae* *MNN9* gene was described previously (Choi et al. *Proc Natl Acad Sci U S A*. 2003 Apr 29;100(9):5022-7). In a similar fashion, the rat GnTII gene was cloned. The rat GnTII gene (GenBank accession number U21662) was PCR amplified using Takara *EX Taq*TM polymerase (Panvera) from rat liver cDNA library (Clontech) with RAT1 (5'-TTCCTCACTGCAGTCTTCTATAACT-3', **SEQ ID NO: 116**) and RAT2 (5'-TGGAGACCATGAGGTTCCGCATCTAC-3', **SEQ ID NO: 117**) primers. The PCR product was then cloned into pCR2.1-TOPO vector (Invitrogen) and sequenced. Using this vector as a template, the *AscI*-*PacI* fragment of GnTII, encoding amino-acids 88-443, was amplified with *Pfu Turbo* polymerase (Stratagene) and primers, RAT44 and RAT11 (5'-TTGGCGCGCCTCCCT AGTGTACCAGTTGAACTTTG-3' (**SEQ ID NO: 118**) and 5'-GATTAATTAACCTCACTGCAGTCTTCTATAACT -3' (**SEQ ID NO: 119**) respectively, introduced *AscI* and *PacI* restriction sites are underlined). Following confirmation by sequencing, the catalytic domain of rat GnTII was then cloned downstream of the *PMA1* promoter as a *AscI*-*PacI* fragment in pBP124. In the final step, the gene fragment encoding the *S. cerevisiae* *Mnn2* localization signal was cloned from pJN281 as a *NotI*-*AscI* fragment to generate an in-frame fusion with the catalytic domain of GnTII, to generate plasmid pTC53.

Please delete the paragraph on page 158, lines 11-14, and replace it with the following paragraph:

SEQ ID NO: 5

Class 2 mannosidase conserved amino acid sequence:

Appln. No. 10/616,082

Preliminary Amendment dated Jan. 16, 2004

Preliminary Amendment In Response To Notice To File Missing Parts Dated 10/16/03

Leu Lys Val Phe Val Val Pro His Ser His Asn Asp Pro Gly Trp Ile Gln Thr Phe Glu Glu Tyr
~~Try~~ Tyr